

A further search for selective antagonists at M₂-muscarinic receptors

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1 In an attempt to obtain more selective antagonists acting at muscarinic M₂-receptors, analogues of 4-diphenylacetoxy-N-methylpiperidine methobromide (4-DAMP methobromide) have been synthesized. These were tested, along with silabenzhexol, procyclidine, sila-procyclidine and AFDX-116, in dose-ratio experiments with guinea-pig isolated atria at 30°C and ileum at 30°C and 37°C. The agonist was carbachol and the selectivity was assessed from the difference between log K for receptors in ileum and log K for receptors in atria.

2 The selectivity was not related to the affinity and some weakly active compounds retained appreciable selectivity but no compound had greater selectivity than 4-DAMP methobromide or pentamethylene bis-(4-diphenylacetoxy-N-methylpiperidinium) bromide.

3 Structure-activity relations are discussed. There seem to be steric limits to affinity but there are no obvious indications of the structural features associated with selectivity. It is suggested that more selective drugs may be obtained by introducing groups which may reduce affinity.

Introduction

Metho-salts of 4-diphenylacetoxy N-methylpiperidine (4-DAMP methiodide or methobromide) have greater affinity for muscarinic receptors in guinea-pig ileum than for those in guinea-pig atria (Barlow *et al.*, 1976). If two molecules are linked together with a pentamethylene chain a compound (bis-5 4-DAMP bromide) is obtained which is weaker but more selective (Barlow & Shepherd, 1985). In this paper the results obtained in a further search for compounds which differentiate between muscarinic receptors in guinea-pig ileum and guinea-pig atria are presented. It includes tests made with other compounds reported to be selective, such as sila-procyclidine (Mutschler & Lambrecht, 1984) and AFDX-116 (Hammer *et al.*, 1986; Giraldo *et al.*, 1986).

Methods

Guinea-pig isolated ileum

The guinea-pig ileum was set up as described by Edinburgh Staff (1974) with the responses recorded isotonicity and a load of about 0.5 g. The agonist, carbachol, was allowed to act for 30 s and added once every 90 s by relays controlled from a PET microcomputer. The tissue was suspended in Krebs solution

(Edinburgh Staff, 1974) aerated with a mixture of 95% O₂ and 5% CO₂, usually containing norphenylephrine, 5 µM (see below), and, as in previous work (Barlow *et al.*, 1976), experiments were done at 29.8 ± 0.3°C and 37.0 ± 0.1°C. The lower temperature was needed for comparisons with results on atria and the effect of temperature on dose-ratio should give some indication of the enthalpy of binding of the antagonist.

Alternate small and large control responses were obtained, usually to 0.1 and 0.2 µM carbachol. When these were regular the tissue was exposed to a solution of the antagonist and the concentration of agonist was increased to try to obtain responses which roughly matched the controls. When these were regular the approximate dose-ratio is given by the ratio of the concentrations of agonist used in the presence and in the absence of the antagonist and an exact dose-ratio was calculated from the size of the responses by a calculation similar to a 4-point assay (Edinburgh Staff, 1974; Barlow, 1983). Usually a second concentration of antagonist was tested on each preparation and where possible a fresh set of control responses was obtained.

Guinea-pig isolated atria

The atria were set up in Krebs solution (Edinburgh Staff, 1974) aerated with a mixture of 95% O₂ and 5%

¹ Correspondence.

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ylated, to give compounds 10 and 11. The bromine is labile and when the bromo-ester is allowed to stand in methanol the methoxy ester is obtained which was methylated to give compound 9.

Diphenylpropionic acid, diphenyl- α -methylacetic acid, cyclohexylphenyl acetic acid, anthracene-9-carboxylic acid, adamantane-1-carboxylic acid and triphenylacetic acid were converted to their acid chlorides, and then esterified with 4-hydroxy-N-methylpiperidine and the bases methylated, to give compounds 3, 8, 16, 19, 23 and 25. Anthracene-9-carboxylic acid was reduced to the dihydro-compound with sodium and alcohol and this was converted to the acid chloride, esterified and methylated to give compound 20. Di-(2-thiophenyl)acetic acid was prepared from the reaction of thiophene (2 mol) with chloral hydrate in concentrated H₂SO₄ (Freeman *et al.*, 1948) and hydrolysing the 1,1-dithiophenyl-3,3,3-trichloroethane formed to obtain the substituted acetic acid. This was converted into compound 17 via the acid chloride.

Compound 4 was prepared from diphenylacetyl chloride and 4-hydroxy tetrahydro-thiopyrane, obtained from reduction of tetrahydro thiopyran-4-one with NaBH₄. The ester reacted readily with methyl iodide to give the sulphonium compound.

The ethyl and *n*-propyl homologues of 4-DAMP, compounds 5 and 6, were obtained by treating the base, 4-diphenylacetoxy-N-methylpiperidine with ethyl- and *n*-propyl- bromides, respectively. The hydroxyethyl derivative, compound 7, was obtained from the secondary amine, 4-diphenylacetoxy piperidine, which was prepared by catalytic debenzoylation of the N-benzyl compound. The secondary amine was treated with ethylene bromhydrin and then quaternized with methyl bromide.

The *o*-, *m*-, and *p*-methyl- and *p*-phenyl compounds (12, 13, 14 and 24) were obtained from the corresponding benzophenones which were treated with sodium and carbon dioxide (Hamrick & Hauser, 1959) to give the diphenyl-glycollic acid and then with red phosphorus and HI to reduce out the hydroxyl group and give the substituted phenylacetic acid. This was converted into the acid chloride, esterified and methylated. In a similar way dibenzosuberone was converted into the dibenzo-cycloheptane carboxylic acid and on to give compound 22. With thioxanthene-9-one, however, the method of Heacock *et al.* (1958) was used: the keto group was reduced to -CH₂- with sodium and alcohol and this was treated with butyllithium followed by carbon dioxide. The acid was then converted into the acid chloride, esterified and methylated to obtain compound 21.

The di-*o*-chloro compound (15) was obtained by converting *o*-chlorobenzaldehyde to the benzoin, benzil, benzilic acid and phenylacetic acid (cf. the preparation of the di-*p*-tolyl compound, Barlow & Shepherd, 1985).

The diphenylacetyl amide (compound 26) was the sample described by Barlow *et al.* (1978).

Compounds

Carbachol chloride was obtained from Sigma and norphenylephrine hydrochloride from Aldrich. The following compounds were gifts for which we are most grateful: (\pm)-sila-benzhexol from Dr R.M. Eglen, Syntex Research Centre, Edinburgh; (\pm), (-) and (\pm) sila-procylidine from Dr G. Lambrecht, Department of Pharmacology, Frankfurt and AFDX-116 (11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5, 11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepin-6-on) from Boehringer Ingelheim.

Results

The results are summarized in Table 2. The dose-ratios for each concentration have been used to calculate the affinity constant and the mean estimate of log affinity constant (K) is shown (\pm s.e.). The asterisk indicates experiments done in the presence of norphenylephrine and the two asterisks indicate the *p*-methyl compound tested with and without norphenylephrine (for which the dose-ratios are given in Table 1). If the compound behaves competitively the mean estimate of log K should be the same at all concentrations: this seems to be true for the range of concentrations studied. Likewise there are no obvious differences between results obtained using effects on atrial rate and those obtained using effects on atrial force.

The overall mean values for effects of all concentrations on the ileum at 30°C and for all concentrations on atrial rate and atrial force have been used to prepare Figure 1, in which the compounds are indicated by their number in Table 2 and the horizontal line indicates the selectivity, being the difference between log K for ileum and log K for atria. (For compounds 5 and 6 log K for ileum at 37°C was used because measurements were not made on ileum at 30°C.) The compounds X1 to X6 are included for comparison: X1 is 4-diphenylacetoxy-N-methylpiperidine hydrochloride (4-DAMP not quaternized) and X2 is the benzilic ester of 4-hydroxy-N-methylpiperidine methiodide (with hydroxyl in place of hydrogen attached to the acetyl group). The results for these compounds are taken from Barlow *et al.* (1976). The results for the other compounds are given by Barlow & Shepherd (1985): X3 is *p*-dimethyl-4-DAMP methiodide, X4 is the *p*-dichloro-compound: X5 and X6 are, respectively, the fluorene-9-carboxy- and xanthene-9-carboxy- analogues.

Note that with all the compounds except AFDX-116 (shown as a broken line) the affinity for the ileum is greater than that for the atria.

Table 2 Log affinity constants

Conc (μ M)	Rate	Atria	Force	30°C	Ileum	37°C
I* (±)-Sila-benzhexol						
0.1	7.64 ± 0.04 (4)		7.60 ± 0.12 (4)	8.24 ± 0.05 (4)		8.19 ± 0.04 (4)
0.5	7.66 ± 0.08 (4)		7.50 ± 0.07 (4)	8.19 ± 0.08 (4)		8.20 ± 0.04 (4)
II* (±)-Procyclidine						
0.5	7.08 ± 0.09 (5)		7.06 ± 0.06 (5)	7.77 ± 0.03 (6)		7.78 ± 0.02 (6)
2.0	7.10 ± 0.10 (5)		7.09 ± 0.05 (5)	7.74 ± 0.05 (5)		7.78 ± 0.02 (5)
III* (-)-Silaprocyclidine						
0.1	7.43 ± 0.26 (3)		7.52 ± 0.07 (3)	8.43 ± 0.06 (3)		8.39 ± 0.03 (3)
IV* (+)-Silaprocyclidine						
0.1	7.56 ± 0.06 (3)		7.46 ± 0.04 (3)	8.31 ± 0.07 (3)		8.07 ± 0.06 (3)
V* AFDX-116						
1.0	7.26 ± 0.14 (2)		7.27 ± 0.04 (2)	6.15 ± 0.03 (2)		6.14 ± 0.12 (2)
2.0	7.36 ± 0.14 (3)		7.31 ± 0.05 (3)	6.22 ± 0.06 (3)		6.12 ± 0.04 (3)
10.0	7.35 ± 0.14 (3)		7.27 ± 0.09 (3)	6.16 ± 0.04 (3)		6.06 ± 0.08 (3)
1 Reversed 4-DAMP MeBr (-O-CO- replaced by -CO-O-)						
0.1	7.06 ± 0.07 (3)		7.24 ± 0.16 (3)	7.81 ± 0.13 (3)		7.85 ± 0.15 (3)
0.5	7.26 ± 0.08 (3)		7.35 ± 0.12 (3)	7.77 ± 0.17 (3)		7.85 ± 0.15 (3)
2 4-Diphenylmethoxy-N-methylpiperidine MeBr (short 4-DAMP ether)						
0.1	7.60 ± 0.12 (3)		7.69 ± 0.09 (3)	8.01 ± 0.04 (4)		8.02 ± 0.07 (4)
0.5	7.78 ± 0.12 (3)		7.79 ± 0.08 (3)	7.98 ± 0.06 (4)		8.03 ± 0.04 (4)
3 4-Diphenylpropionyl-N-methylpiperidine MeBr (homo-4-DAMP)						
5.0	5.61 ± 0.23 (4)		5.74 ± 0.12 (4)	6.26 ± 0.03 (4)		6.20 ± 0.12 (4)
10.0	5.65 ± 0.19 (4)		5.71 ± 0.09 (4)	6.23 ± 0.04 (5)		6.21 ± 0.10 (5)
50.0	5.61 ± 0.06 (3)		5.61 ± 0.07 (3)	6.21 ± 0.02 (4)		6.13 ± 0.09 (4)
4* Sulphonium analogue of 4-DAMP MeBr						
0.1	7.42 ± 0.18 (4)		7.48 ± 0.06 (4)	7.86 ± 0.05 (4)		7.78 ± 0.03 (4)
0.5	7.35 ± 0.13 (4)		7.48 ± 0.03 (4)	7.88 ± 0.04 (4)		7.82 ± 0.03 (4)
5 4-Diphenylacetoxy-N-methylpiperidine EtBr						
0.1	7.82 ± 0.13 (4)					8.67 ± 0.05 (4)
0.5	7.70 ± 0.05 (3)					8.65 ± 0.02 (4)
2.0						8.70 ± 0.03 (4)
6 4-Diphenylacetoxy-N-methylpiperidine n-PrBr						
0.5	6.49 ± 0.10 (4)					7.41 ± 0.05 (4)
2.0						7.35 ± 0.02 (4)
7 4-Diphenylacetoxy-N-methylpiperidine-hydroxyethyl bromide						
0.1	7.43 ± 0.06 (3)		7.20 ± 0.03 (4)	7.88 ± 0.03 (3)		7.91 ± 0.01 (4)
0.5	7.38 ± 0.05 (3)		7.31 ± 0.03 (4)	7.79 ± 0.05 (3)		7.91 ± 0.04 (4)
8* 4-Diphenyl-methyl-acetoxy-N-methylpiperidine MeBr (α-methyl 4-DAMP)						
0.01	9.25 ± 0.08 (4)		9.22 ± 0.09 (4)	9.68 ± 0.03 (4)		9.50 ± 0.08 (4)
0.02	9.33 ± 0.13 (2)		9.46 ± 0.13 (2)	9.62 ± 0.01 (2)		9.70 ± 0.21 (2)
0.05	9.32 ± 0.04 (3)		9.28 ± 0.06 (3)	9.77 ± 0.05 (3)		9.57 ± 0.06 (3)
0.1	9.26 ± 0.06 (3)		9.40 ± 0.10 (3)	9.64 ± 0.02 (3)		9.62 ± 0.09 (3)
9* 4-Diphenyl-methoxy-acetoxy-N-methylpiperidine MeBr (α-methoxy 4-DAMP)						
0.1	7.77 ± 0.09 (4)		7.62 ± 0.09 (4)	8.20 ± 0.02 (4)		8.16 ± 0.03 (4)
0.5	8.02 ± 0.10 (3)		8.02 ± 0.12 (3)	8.28 ± 0.04 (4)		8.25 ± 0.01 (4)
10 4-Diphenyl-chloro-acetoxy-N-methylpiperidine MeBr (α-chloro 4-DAMP)						
0.02	9.48 ± 0.10 (4)		9.42 ± 0.04 (4)	9.39 ± 0.11 (4)		9.31 ± 0.05 (4)
0.1	9.47 ± 0.11 (4)		9.38 ± 0.05 (4)	9.54 ± 0.25 (4)		9.69 ± 0.06 (4)
11 4-Diphenyl-bromo-acetoxy-N-methylpiperidine MeBr (α-bromo 4-DAMP)						
0.02	9.26 ± 0.11 (4)		9.20 ± 0.17 (4)	9.30 ± 0.02 (6)		9.32 ± 0.04 (6)
0.1	9.26 ± 0.07 (4)		9.21 ± 0.06 (4)	9.51 ± 0.07 (6)		9.45 ± 0.04 (6)
12* (±)-Mono-o-methyl-4-DAMP MeBr						
0.1	7.25 ± 0.14 (2)		7.30 ± 0.06 (3)	7.46 ± 0.13 (3)		7.50 ± 0.09 (3)
0.5	7.24 ± 0.15 (2)		7.28 ± 0.01 (3)	7.42 ± 0.10 (3)		7.47 ± 0.10 (3)
13* (±)-Mono-m-methyl-4-DAMP MeBr						
0.1	7.40 ± 0.11 (5)		7.57 ± 0.06 (5)	8.07 ± 0.04 (5)		7.99 ± 0.03 (5)
0.5	7.44 ± 0.08 (5)		7.58 ± 0.06 (5)	8.07 ± 0.04 (5)		7.96 ± 0.03 (5)

Table 2 Log affinity

Conc (μ M)
14** (±)
0.1
0.5
15* o-Di
0.1
0.5
16 Phenyl
0.1
0.5
17 4-Di-
0.1
0.25
18 9-Hyd
0.1
0.5
19 4-(Ani
2.0
10.0
20 4-(9,10
0.1
0.5
21* 4-(Th
0.1
0.5
22* 4-(Di
0.5
5.0
23* 4-(Ad
1.0
5.0
24* (±)-N
1.0
5.0
25 4-Triph
5.0
26 2-Dipha

* Indicates experiments
norphenylephrine and
† Tested up to 10 μ M:

Discussion

In this work there appear to be compounds which have greater affinity for receptors in ileum than in atria but A selectivity cannot be an a made. There is considerable degree of selectivity (Barlow & Shepherd, 1978) perhaps not surprising from one study to a benzhexol and sila-proc cyclidine than has been found

Table 2 Log affinity constants

Conc (μ M)	Atria Rate	Force	Ileum 30°C	37°C
14** (\pm)-Mono- <i>p</i> -methyl-4-DAMP MeBr				
0.1	7.81 \pm 0.10 (4)	7.69 \pm 0.11 (4)	8.17 \pm 0.03 (4)	8.15 \pm 0.03 (4)
0.5	7.77 \pm 0.07 (4)	7.70 \pm 0.11 (4)	8.12 \pm 0.05 (4)	8.16 \pm 0.02 (4)
15* <i>o</i> -Dichloro-4-DAMP MeBr				
0.1	7.44 \pm 0.08 (4)	7.36 \pm 0.07 (4)	7.74 \pm 0.02 (4)	7.68 \pm 0.01 (4)
0.5	7.39 \pm 0.02 (4)	7.40 \pm 0.03 (4)	7.66 \pm 0.03 (4)	7.67 \pm 0.02 (4)
16 Phenyl-cyclohexylacetoxy-N-methylpiperidine MeI (cyclohexyl analogue)				
0.1	8.61 \pm 0.01 (3)	8.50 \pm 0.05 (3)	9.07 \pm 0.09 (3)	9.36 \pm 0.05 (3)
0.5	8.50 \pm 0.03 (3)	8.42 \pm 0.03 (3)	9.06 \pm 0.07 (3)	9.33 \pm 0.01 (3)
17 4-Di-2-thiophenylacetoxy-N-methylpiperidine MeBr				
0.1	8.97 \pm 0.07 (4)	8.92 \pm 0.05 (5)	9.32 \pm 0.07 (5)	9.31 \pm 0.03 (5)
0.25	9.03 \pm 0.04 (3)	9.13 \pm 0.13 (3)	9.16 \pm 0.03 (5)	9.22 \pm 0.04 (5)
18 9-Hydroxyfluorene ester of N-methyl- <i>iso</i> -nipecotic acid MeBr (reversed ester -O-CO- replaced by -CO-O-)				
0.1			7.35 \pm 0.17 (4)	7.22 \pm 0.09 (4)
0.5	7.03 \pm 0.08 (3)	6.81 \pm 0.08 (3)	7.29 \pm 0.15 (4)	7.05 \pm 0.11 (3)
19 4-(Anthracene-9-carboxy)-N-methylpiperidine MeBr				
2.0	6.67 \pm 0.11 (4)	6.61 \pm 0.08 (4)	6.87 \pm 0.08 (4)	6.87 \pm 0.08 (6)
10.0	6.67 \pm 0.13 (3)	6.44 \pm 0.05 (2)	6.93 \pm 0.09 (4)	6.89 \pm 0.08 (5)
20 4-(9,10-Dihydroanthracene-9-carboxy)-N-methylpiperidine MeBr				
0.1	8.04 \pm 0.06 (4)	7.98 \pm 0.05 (4)	8.51 \pm 0.06 (4)	8.44 \pm 0.08 (4)
0.5	7.89 \pm 0.10 (3)	7.69 \pm 0.06 (2)	8.45 \pm 0.08 (4)	8.46 \pm 0.10 (4)
21* 4-(Thioxanthen-9-carboxy)-N-methylpiperidine MeBr				
0.1	7.87 \pm 0.14 (4)	7.74 \pm 0.10 (4)	7.81 \pm 0.02 (4)	7.81 \pm 0.04 (4)
0.5	7.66 \pm 0.06 (3)	7.66 \pm 0.05 (3)	7.74 \pm 0.04 (3)	7.70 \pm 0.01 (3)
22* 4-(Dibenzocycloheptane-carboxy)-N-methylpiperidine MeBr (ethane-bridged 4-DAMP)				
0.5	6.12 \pm 0.17 (3)	5.93 \pm 0.42 (5)	6.27 \pm 0.02 (5)	6.35 \pm 0.05 (4)
5.0	6.16 \pm 0.01 (2)	6.13 \pm 0.06 (4)	6.24 \pm 0.02 (4)	6.34 \pm 0.04 (4)
23* 4-(Adamantane-1-carboxy)-N-methylpiperidine MeBr				
1.0	5.85 \pm 0.08 (4)	5.94 \pm 0.04 (4)	6.65 \pm 0.05 (4)	6.58 \pm 0.08 (4)
5.0	5.88 \pm 0.08 (4)	5.92 \pm 0.04 (4)	6.62 \pm 0.04 (4)	6.61 \pm 0.06 (4)
24* (\pm)-Mono- <i>p</i> -phenyl 4-DAMP MeBr				
1.0	5.94 \pm 0.06 (3)	5.87 \pm 0.11 (3)	6.28 \pm 0.03 (4)	6.08 \pm 0.08 (4)
5.0	6.02 \pm 0.05 (3)	5.81 \pm 0.02 (3)	6.26 \pm 0.04 (4)	6.15 \pm 0.04 (4)
25 4-Triphenylacetoxy-N-methylpiperidine MeBr				
5.0	5.60 \pm 0.13 (3)	5.71 \pm 0.04 (3)	6.41 \pm 0.04 (3)	6.36 \pm 0.01 (3)
26 2-Diphenylacetamido-N-ethyltrimethylamine ethiodide†				

*Indicates experiments done in the presence of norphenylephrine, 5 μ M. **Compound tested with and without norphenylephrine and the results are shown in Table 1.

† Tested up to 10 μ M: dose-ratio on ileum and atria about 5: log K < 5.6.

Discussion

In this work there appear to be several compounds which have greater affinity for muscarinic receptors in ileum than in atria but AFDX-116 clearly has greater affinity for receptors in atria than in ileum, so selectivity cannot be an artifact of the way the tests are made. There is considerable uncertainty about the degree of selectivity (Barlow & Shepherd, 1985) and it is perhaps not surprising that estimates of selectivity vary from one study to another. Our results with sila-benzhexol and sila-procyclidine indicate less selectivity than has been found in other work but it is clear,

for instance, that sila-procyclidine (III) has greater activity and selectivity than procyclidine (II).

Selectivity does not appear to be associated with any particular degree of affinity: there are potent compounds with low selectivity and weak compounds with appreciable selectivity. The distribution of selectivity in Figure 1 can be explained by supposing that the receptors in atria and ileum are not identical. Unless a simpler explanation can be found it seems reasonable to seek to interpret the results by looking at the structures of the compounds to try to see if these suggest differences between the receptors with which the compounds interact.

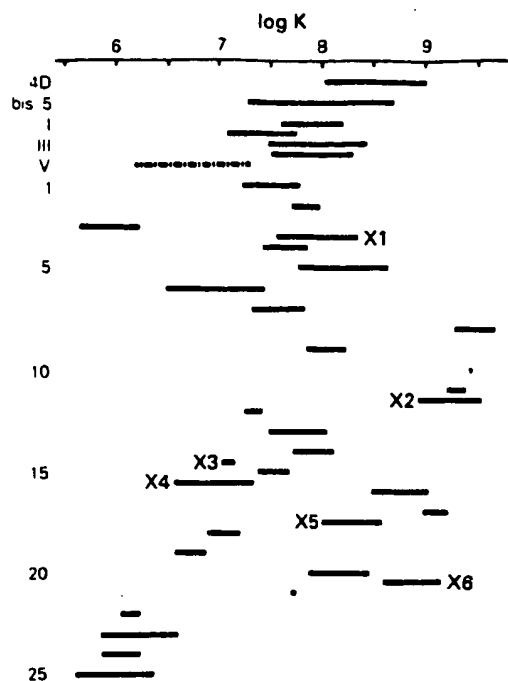


Figure 1 The log affinity constant (K : at 30°C in Krebs solution with carbachol as agonist) is shown for the compounds, numbered according to the list in Table 2. The compound at the top is 4-DAMP MeBr (4D) and results for pentamethylene bis-4-DAMP Br (bis-5) are shown below. The line indicates the selectivity: it is the difference between log K for the ileum and log K for the atria. With all the compounds except AFDX-116 (compound V, broken line) the value for the ileum is greater than that for the atria. Results for the compounds X1 to X6 are included for comparison: X1 is the tertiary compound (4-DAMP HCl), X2 is the benzilic ester of 4-hydroxy-N-methyl piperidine methiodide; X3 is di-*p*-methyl-4-DAMP MeBr; X4 is di-*p*-chloro- 4-DAMP MeBr; X5 and X6 are the fluorene-9-carboxy- and xanthene-9-carboxy- esters, respectively.

The general aim (limited by what it is possible to make) has been to investigate the effects of changing the ester group and chain length (compounds 1–3), changing the onium group (compounds 4–7), changing the α -hydrogen (compounds 8–11), substitution of the benzene ring (compounds 12–15 and 24), replacing a benzene ring (compounds 16 and 17) and linking the benzene rings together (compounds 18–23).

The extra methylene group in homo-4-DAMP (3) drastically reduces affinity but the compound retains some selectivity. Replacement of quaternary ammonium by tertiary sulphonium (4) reduces affinity and selectivity. If the compound is compared with 4-

DAMP HCl (X1) it seems as if the loss of a methyl group has reduced affinity but the change of the atom from nitrogen to sulphur has reduced selectivity. Alkylation of the nitrogen also reduces affinity but not as drastically (5–7). Affinity is appreciably increased by α -methyl-, chloro- or bromo- groups (8, 10 and 11) but reduced by α -methoxy (9). This is surprising because the compounds are at least as active as the α -hydroxy compounds (the benzilic ester, X1) and it is usually assumed that the high affinity of hydroxy compounds can be ascribed to hydrogen bonding with the receptor, which cannot be true for methyl-, chloro- or bromo- compounds. A comparison of the crystal structures, however, shows that when the piperidine rings are superimposed, the hydroxyl group in the tropic acid part of (–)-hyoscyne methiodide is in a very different position from the hydrogen on the acetyl part of 4-DAMP methiodide (Barlow, Howard, Johnson and Sanders, unpublished).

Either chloro- or methyl- substituents in the benzene ring reduce activity. The series of compounds 12, 13 and 14 suggests that a methyl group disturbs binding most in the *o*-position (12) and least in the *p*-position (14), but even in this position the compound is not as active or as selective as the unsubstituted 4-DAMP methobromide. The di-*p*-methyl- compound (X2) is even weaker than the mono-*o*-methyl compound (12). In contrast the di-*o*-chloro- compound (15) is more active than the di-*p*-chloro- compound (X3), which retains some selectivity, but both are much weaker than 4-DAMP methobromide.

Replacement of one benzene ring by cyclohexyl increases affinity (16) but reduces selectivity and replacement of two benzene rings by thiophene (17) increases affinity and reduces selectivity even further.

The reversed ester of fluorene (18) should be compared with the fluorene ester (X4) and both should be compared with 4-DAMP methobromide and its reversed ester (1). Reversing the ester group reduces affinity and selectivity more with the fluorene compounds, even though these are weaker. The anthracene compound (19) is remarkably weak but reduction to the 9,10-dihydro-compound (20) increases affinity almost 100 fold. The thioxanthene ester (21) should be compared with its oxygen analogue (X5) and the dihydro-anthracene compound (20) should be compared with the cycloheptane analogue (22). These results all reinforce the idea that there are steric constraints to the binding of this part of the molecule. If it is very flat (19) or bulky (22) it cannot bind well. It is therefore not surprising that the adamantyl ester (23), *p*-phenyl 4-DAMP MeBr (24) and the triphenylacetic ester (25) are weak.

It is not clear, however, the extent to which electronic effects are involved in binding nor what combination of steric and electronic effects determine selectivity. It seems possible that, at this stage,

progress in the development may be made by making affinity, in the hope that at one type of receptor worked with the amide (compound AFDX-116, *in vivo*, is also remarkably weak).

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progress in the development of selective compounds may be made by making changes likely to reduce affinity, in the hope that the reduction will be greater at one type of receptor than the other. This has not worked with the amide (26) but it is notable that the compound AFDX-116, which is undoubtedly selective, is also remarkably weak compared with atropine.

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